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CALIFORNIA GREEN LACEWING FLY

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INTRODUCTION

The green lacewing fly (*Chrysopa californica* Coquillett) (fig. 1) has been observed by the writer on many occasions during the past five years in connection with outbreaks of aphids in southern Arizona and California, and at various times the extreme usefulness of the species in controlling these outbreaks has been noted. An opportunity for making

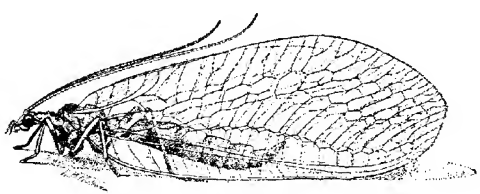

FIG. 1.—The California green lacewing fly (*Chrysopa californica*): Adult.

a complete study of the species came to hand during the past year (1915), and this paper is prepared for the purpose of recording the facts as observed and interpreted during this study.

HISTORICAL REVIEW

This lacewing fly has been known since 1890, when it was collected in California and described by Coquillett (3)[1] in the Report of the State Board of Horticulture of that State. The next reference (1, p. 156) to it is in Banks's Revision of the Nearctic Chrysopidae. In this paper, published in 1903, Mr. Banks redescribed the species and stated that "it is the most abundant species on the Pacific coast."

It was not, however, until the year 1912 that the real usefulness and economic value of this species was brought forth. In this year

[1] Reference is made by number to "Literature cited," p. 524.

Quayle (7) listed it as an enemy of the Citrus red spider (*Tetranychus mytilaspidis* Riley) and remarked that "this is the commonest of the predatory insects occurring on citrus trees." Two years later the species was mentioned as an enemy of the Citrus red spider by Ewing (6). Essig (4), in 1911, described it briefly and included a few remarks on its habits and hosts. In 1913 (5), in his "California Insects," he credited this species of Chrysopa with feeding upon 14 different species of insects.

In August (2), 1915, and again in October (2), Mr. E. J. Branigan, a deputy of the California Horticultural Commission, referred to the economic importance of this species. In the first citation he reported the insect as feeding upon the "elm-leaf cluster louse." He stated: "Large numbers of the egg clusters of *Chrysopa californica* were present, the larvæ upon hatching, burrowing into the leaf clusters and feeding upon the lice." In October he reported this lacewing larva as attacking a citrus mealy bug (*Pseudococcus* sp.), and states further: "The green lacewing was found to be heavily parasitized by several species of parasites."

DISTRIBUTION OF THE FLY

From our present knowledge of the species it is distinctly of western distribution, occurring throughout the Pacific Coast States, Texas, Arizona, New Mexico, Nevada, Lower California, and doubtless Utah. As early as 1903, Banks (1) stated:

> I have seen specimens from many places; Los Angeles, Tehama, Wanona [Wawona?], San Bernardino, Palo Alto, San Mateo County, Santa Clara County and Siskiou [Siskiyou ?] County, mostly in July and August, but some in April; also from Hood River, Oregon, September; Pullman, Wash., July and August; and King's Canon, Ormsby County, Nevada, July.

Mr. C. N. Ainslie, of the Bureau of Entomology, has taken a specimen of *Chrysopa* sp. at Salt Lake City, Utah, which is without much doubt this species. The writer has taken or seen specimens in southern California, Lower California, Mexico, Arizona, New Mexico, and in many different localities in these States at elevations varying from sea level to 7,000 feet.

HOST INSECTS

While the larvæ of this lacewing fly, as well as Chrysopidae in general, feed primarily upon aphids, their good work is far from being restricted to this group of insects. Mites, leafhoppers, thrips, and doubtless many other insects sufficiently small to be easily captured and devoured are likewise eaten.

Essig (5) has shown the following 14 species of insects to be attacked by *Chrysopa californica:*

Clover mite (*Bryobia pratensis* Garman).
Two-spotted mite (*Tetranychus mytilaspidis* Riley).
Red spider (*T. telarius* Linnaeus).
Apple leafhopper (*Empoasca mali* LeBaron).

Grape leafhopper (*Typhlocyba comes* Say).
The pear Psylla (*Psylla pyricola* Foerster).
Mealy plum plant louse (*Hyalopterus arundinis* Fabricius).
Melon aphis (*Aphis gossypii* Glover).
Black peach aphis (*Aphis persicæ-niger* Erwin Smith).
Green Citrus plant louse (*Macrosiphum citrifolii* Ashmead).
Citrus mealy bug (*Pseudococcus citri* Risso).
Frosted scale (*Eulecanium pruinosum* Coquillett).
Red scale (*Chrysomphalus aurantii* Maskell).
Purple scale (*Lepidosaphes beckii* Newman).

Mr. E. G. Smyth, working at Tempe, Ariz., found larvæ of *C. californica* feeding also on the wheat thrips (*Euthrips tritici* Fitch), which they apparently preferred to the pea aphis (*Macrosiphum pisi* Kaltenbach). Mr. R. N. Wilson, also at the Tempe laboratory, observed larvæ of *C. californica* feeding upon the barley mite (*Notophallus viridis* Banks) and on the "green bug" (*Toxoptera graminum* Rondani), while the writer reared the species exclusively on the corn leaf aphis (*Aphis maidis* Fitch), it being a very important check upon this pest.

LIFE HISTORY AND HABITS OF THE LACEWING FLY

THE ADULT

As before stated, this species of Chrysopa was first described by Coquillett (3) in 1890, and later (in 1903) redescribed by Banks (1). The original description of the adult by Coquillett is as follows:

> Pale green, a yellowish white dorsal stripe extends from front of thorax to tip of abdomen; front of head whitish; an irregular wine-red stripe extends from each eye to the mouth, and on its hind border, next the eye, is a black streak; front corners of thorax marked with black. Antennæ pale yellowish, minutely ringed with white. Wings greenish hyaline, obtusely pointed at their tips; veins and veinlets wholly green; seven or eight of the veinlets along the hind edge of front wings before the tips are forked; stigma somewhat opaque, yellowish green; legs green, tarsi whitish, the tips brown. Eyes greenish golden, becoming glaucous brown after death. In dried specimens the green coloring becomes more yellowish and the tarsi assumes a slightly darker color than the tibiae. Length 9 to 10 mm. (about ⅜ of an inch); expands from 24 to 28 mm. (about one inch or slightly over).

The adults are delicate green, flitting creatures which dart up from the shady protecting vegetation as one walks along a fence row or through an alfalfa field. The males are slightly smaller than the females and appear more vivid in color. During the breeding season both are short-lived. Neither sex has ever been noted by the writer to feed in the adult stage, even when food was offered, and doubtless all of the lacewing flies take little or no food in this period of their existence.

Copulation takes place almost immediately after the adults have issued and become dry, and in all cases under observation the male was dead on the following day. Oviposition usually begins the day following copulation and may continue for a period of three or four days, or the full complement of eggs may be deposited in a single day. Four

females under observation (see Table I) laid an average of 30¾ eggs each, the record being 34, 25, 38, and 26 eggs, respectively. The females, after performing what is apparently their sole purpose in life, die within 24 to 36 hours after oviposition is completed. The adults are especially numerous in southern Arizona during February, March, April, and May, and again during October and November.

THE EGG

The egg (fig. 2) is placed on a long stalk or pedicel, which is hair-like and about half an inch in length. The egg itself is oblong and very small; at first it is whitish, but in a day or two it darkens and thereafter until it hatches the segmentation of the developing larva is revealed through the eggshell. It has a button or lid at the upper end, which is slightly flattened, while the lower end tapers until it is barely larger than the stalk to which it is attached. The original description by Coquillett (3) is as follows:

FIG. 2.—*Chrysopa californica*: Eggs.

Very pale blue, elongate-ovate, pointed at the base, the apex flattened and in its center is a white button-shaped object; surface minutely granulated; length, three and one-half hundredths of an inch; mounted on a bristle-like pedicle from thirteen to eighteen hundredths of an inch long.

The egg stage (see Table I) was found to vary, being from 6 to 12 days in duration under the temperatures at which the experiment was carried on. The average time required for the 122 eggs under observation was 8 days.

TABLE I.—*The egg stage of Chrysopa californica at Tempe, Ariz., in 1915*

Female No.	Cage No.	Eggs.				Length of stage.	Average mean temperature.
		Date laid.	Number.	Date hatched.	Number.		
						Days.	°F.
1	T 78	Feb. 11	11	Feb. 23	11	12	53
				do.	1	11	53
	T 70	Feb. 12	7	Feb. 24	6	12	53
				do.	10	11	53
	T 80	Feb. 13	15	Feb. 25	5	12	53
				do.	1	11	53
	T 81	Feb. 14	1	do.	1	11	53
2	T 1159	Oct. 12	19	Oct. 19	19	7	66.5
	T 1160	Oct. 13	6	Oct. 20	6	7	67
	T 1185	do.	20	do.	20	7	67
3	T 1186	Oct. 14	13	do.	13	6	68
	T 1187	Oct. 15	4	Oct. 21	4	6	69
	T 1189	Oct. 16	1	Oct. 23	1	7	70
4	T 1190	do.	15	Oct. 22	14	6	70
	T 1196	Oct. 17	11	Oct. 23	11	6	70.5
Total or average			123		122	8	

THE LARVA

The larva when first hatched (fig. 3) is a delicate, white, nearly colorless object, quite conspicuously hairy and with mandibles which are large in comparison to the size of the body, these being about one-fourth its entire length. Coquillett's (3) description follows:

> Mixed with a yellowish white and pinkish brown, the latter color forming a dorsal line and a series of lateral spots; along each side of the body is a row of yellowish white tubercles; head yellowish white, marked with two diverging black stripes on the top, and with a dusky streak each side, having in its middle a black dot; length, 7 mm. (A little over one-fourth of an inch.)

LARVAL HABITS

The hatching process requires but a few minutes, but the larva rests on the empty eggshell for some time after emergence. When the eggshell becomes dry and hardened, the larva hastily crawls down the supporting egg stalk and eagerly begins searching for food. If small aphids or thrips nymphs are present, it quickly seizes one of these and begins feeding. If only full-grown and large aphids are present, it is more cautious, running in a circle around the tempting and monstrous meal or following the aphid, ever and anon stopping as if to consider whether or not it could safely attack a creature so many times larger than itself. Finally, however, its increasing hunger apparently overcomes all fear and it pounces on its prey. The aphid is lifted bodily off its feet, the lacewing larva all the time crushing, piercing, and sucking its prey. The larvæ of all lacewing flies extract their food from the host by piercing it with their long, powerful mandibles, which are hollow, the internal fluids of the host being rapidly absorbed through them. With abundant food present the larva grows rapidly and quickly takes on a robust appearance.

FIG. 3.—*Chrysopa californica:* First instar.

LARVAL DEVELOPMENT

The larvæ in the course of their development molt twice, which divides the larval period into three instars, with a total length of from 11 to 22 days, depending upon the prevailing temperature, the average length being about 16 days. (See Table II.) During this period from 74 to 160 full-grown aphids were eaten by each larva, the number consumed depending upon the temperature, the larvæ being more active and voracious during warmer weather.

TABLE II.—*Table of molts and instars of Chrysopa californica at Tempe, Ariz.*

PART I. FEBRUARY, 1915. AVERAGE MEAN TEMPERATURE, 54° F.

Cage No.	Date hatched.	Date of first molt.	Length of first instar.	Number of aphids eaten.	Date of second molt.	Length of second instar.	Number of aphids eaten.	Date in cocoon.	Length of third instar.	Number of aphids eaten.	Total number of aphids eaten.	Total length of larval period.
			Days.			*Days.*			*Days.*			*Days.*
78-1	Feb. 24	Mar. 3	8	15	Mar. 10	7	10	Mar. 17	7	43	68	22
78-2	...do....	Mar. 2	7	15	Mar. 9	7	15	...do....	8	59	89	22
78-3	...do....	Mar. 3	8	17	...do....	6	12	...do....	8	61	90	22
78-4	...do....	...do....	8	17	Mar. 11	8	16	...do....	6	50	83	22
78-1	Feb. 24	Mar. 4	8	12	...do....	7	16	...do....	6	47	75	21
78-2	...do....	...do....	8	10	...do....	7	16	...do....	6	51	77	21
78-3	...do....	Mar. 5	9	12	Mar. 12	7	23	...do....	5	39	74	21
80-1	Feb. 25	...do....	8	12	...do....	7	22	...do....	5	49	83	20
80-4	...do....	...do....	8	11	...do....	7	19	Mar. 18	6	74	104	21
80-5	...do....	...do....	8	10	...do....	7	15	Mar. 17	5	56	81	20
80-7	...do....	...do....	8	13	Mar. 11	6	25	...do....	6	62	100	20
82-9	...do....	...do....	8	14	Mar. 13	7	27	...do....	5	56	97	22
82-12	...do....	...do....	8	15	Mar. 21	6	28	...do....	6	63	106	22
82-11	...do....	...do....	8	11	Mar. 12	7	20	...do....	5	56	89	20
80-1	...do....	...do....	8	13	Mar. 15	10	32	[1]Mar. 19	.....	57	101	.....
Average.	.....	.....	8	13[2]	.....	7	20	.....	6	55¾	88	20[2]

PART II. OCTOBER, 1915. AVERAGE MEAN TEMPERATURE, 70° F.

59-2	Oct. 19	Oct. 23	4	14	Oct. 26	3	21	Oct. 30	4	128	143	11
59-3	...do....	...do....	4	17	Oct. 27	4	33	Nov. 1	5	79	129	13
59-1	...do....	...do....	4	16	Oct. 26	3	18	...do....	6	109	143	13
59-2	...do....	...do....	4	15	...do....	3	34	Oct. 31	5	100	147	12
59-7	...do....	...do....	4	13	Oct. 27	4	35	...do....	4	95	143	12
59-9	...do....	...do....	4	12	...do....	3	33	...do....	4	90	135	11
59-10	...do....	...do....	4	15	...do....	4	20	...do....	4	93	128	12
59-11	...do....	...do....	4	16	Oct. 29	3	30	...do....	5	124	170	12
59-12	...do....	...do....	4	14	Oct. 27	4	36	...do....	4	91	141	12
59-13	...do....	...do....	4	14	...do....	4	32	...do....	4	100	136	12
59-14	...do....	...do....	4	12	...do....	4	39	...do....	4	107	158	12
Average.	.....	.....	4	14½	.....	3⅔	31	.....	4½	98	143	12

[1] Died.

From these records it is seen that a *C. californica* larva under natural conditions, eating both large and small aphids, must often consume 300 or 400 of them during the course of its development. The economic value of these larvæ is thus seen to be enormous. It was found that an average of about 14 full-grown adults of *Aphis maidis* were consumed in the first instar, 4 to 7 aphids being eaten the first day after hatching. The duration of the first instar was found to vary with the temperature, it being from 4 to 9 days and the average period about 6 days in length. A great many more aphids are consumed during the second instar than in the first. This instar averages nearly a day shorter, being 7 days during March and 3⅔ days during October, 20 being the average number of aphids eaten by each of 15 larvæ during the former period and 31 during the latter. In actions and habits it is largely the same as the first instar except for the increased power of destroying aphids

The third-instar larvæ (fig. 4), while having a period of life averaging about the same in length as that of the second instar, make up for it in the number of aphids consumed. Fifteen larvæ in March each ate an average of 9½ aphids a day or 55½ during the entire period; whereas 11 larvæ each ate an average of nearly 22 full-grown aphids a day or 98 for the third-instar period, this being nearly twice as many as are eaten during the first and second instars. In Table IV it will be noted that a third-instar larva of *C. californica* in cage 59–14 ate 40 full-grown *Aphis maidis* in one day of 24 hours. The average length of the third instar was 6 days in March and 4½ days in October.

FIG. 4.—*Chrysopa californica*: Third instar.

Tables III and IV show the daily consumption of aphids by 26 larvæ during their entire larval period.

TABLE III.—*Daily feeding record of 15 larvæ of Chrysopa californica at Tempe, Ariz., in February, 1915*[a]

Cage No.	Date hatched.	February.					March.					
		24	25	26	27	28	1	2	3	4	5	6
78–1	Feb. 23	5	5	2	2	0	0	1	*m* 2	3	3	0
78–2	do	4	4	4	1	2	0	*m* 0	5	5	5	0
78–3	do	5	3	5	2	0	0	2	*m* 3	4	5	0
78–4	do	4	5	2	3	0	1	2	*m* 0	3	5	2
79–1	Feb. 24		2	2	2	2	0	0	2	*m* 1	3	5
79–2	do		2	2	3	1	1	0	1	*m* 0	4	8
79–3	do		4	3	0	3	2	0	0	0	*m* 5	5
80–3	Feb. 25			3	4	1	3	1	0	0	*m* 3	4
80–4	do			3	1	4	2	0	0	1	*m* 0	7
80–6	do			2	2	2	3	0	1	0	*m* 4	3
80–7	do			3	2	3	1	0	1	0	*m* 4	8
80–9	do			4	3	1	4	1	0	1	*m* 2	7
80–10	do			4	3	1	4	2	0	0	*m* 5	7
80–11	do			4	2	0	5	2	0	0	*m* 4	6
80–12	do			4	5	0	3	3	0	0	*m* 3	3

Cage No.	Date hatched.	March.														Average mean temperature.
		7	8	9	10	11	12	13	14	15	16	17	18	19	20	°F.
78–1	Feb. 23	2	0	0	*m* 3	7	7	8	7	7	4	*c*				54
78–2	do	0	0	*m* 5	10	10	10	10	8	4	2	*c*				54
78–3	do	0	0	*m* 3	9	10	10	10	10	4	5	*c*				54
78–4	do	5	1	0	0	*m* 5	10	9	8	8	10	*c*				54
79–1	Feb. 24	6	1	0	0	*m* 3	9	9	9	8	9	*c*				54
79–2	do	3	1	0	0	*m* 8	9	10	10	10	4	*c*				54
79–3	do	6	6	0	0	1	*m* 4	10	10	8	7	*c*				54
80–3	Feb. 25	5	7	3	0	0	*m* 3	13	13	13	14	*c*				54
80–4	do	0	8	2	2	0	*m* 0	10	13	13	13	15	*c*			54
80–6	do	5	3	0	0	0	*m* 8	8	12	14	14	*c*				54
80–7	do	7	6	0	0	*m* 0	12	10	13	14	14	*c*				54
80–9	do	6	8	3	1	0	*m* 8	9	12	12	15	*c*				54
80–10	do	8	7	0	1	*m* 0	8	10	13	13	15	*c*				54
80–11	do	2	7	1	0	0	*m* 6	8	12	13	15	*c*				54
80–12	do	7	5	0	4	8	2	0	0	*m* 8	12	12	12	13	*c*	54

[a] *m*, Date of molting; *c*, date of spinning cocoon.

TABLE IV.—*Daily feeding record of 11 larvæ of Chrysopa californica at Tempe, Ariz., in October, 1915.a*

Cage No.	Date hatched.	October.												November.	Average mean temperature.
		20	21	22	23	24	25	26	27	28	29	30	31	1	
															°F.
59–2	Oct. 19	7	7	0	*m* 6	15	0	*m* 28	20	25	25	*c* 20	.....	.....	70
59–3	do	7	7	3	*m* 7	14	13	0	*m* 16	23	28	3	0	*c*	70
59–4	do	5	7	4	*m* 7	11	9	*m* 17	19	22	26	25	0	*c*	70
59–5	do	7	7	1	*m* 7	15	12	*m* 20	18	25	25	22	*c*	.....	70
59–6	do	6	7	0	*m* 7	14	14	0	*m* 20	24	23	28	*c*	.....	70
59–7	do	6	5	2	*m* 7	14	13	0	*m* 10	25	25	30	*c*	.....	70
59–9	do	7	6	2	*m* 6	15	5	0	*m* 19	24	26	24	*c*	.....	70
59–10	do	7	7	2	*m* 7	15	8	*m* 14	20	25	25	30	*c*	.....	70
59–11	do	7	7	0	*m* 7	15	14	0	*m* 20	25	23	23	*c*	.....	70
59–12	do	5	7	2	*m* 6	15	11	0	*m* 29	25	25	30	*c*	.....	70
59–13	do	6	6	0	*m* 7	15	17	0	*m* 19	23	25	40	*c*	.....	70
59–14	do														

[a] *m*, Date of molting; *c*, date of spinning cocoon.

Table III shows the record of 15 larvæ during the month of February and Table IV shows the record of 11 larvæ during the month of October. It will be noted that both the daily and total consumption were much larger during the latter and warmer period than during the former and that the total feeding period was nearly half the length during this period. Only full-grown wingless specimens of *Aphis maidis* were used in this experiment.

FIG. 5.—*Chrysopa californica*: Pupal case.

MOLTING

When the larva gets ready to molt, it settles down in some protected spot and rests for a period of several hours, often a day or more, and when the opportune time seems to have arrived it begins a series of movements, mostly of a rising and falling nature, calculated to burst the skin on the back. When this is finally accomplished, it crawls out and, after a few minutes' rest, is the same voracious creature it was before except only that its size is greater than in the preceding instar.

During the first and second instars, after the larva has eaten its quota of aphids, it rests, often as long as two days; during the last instar, however, this rest period is not apparent, owing to the fact that it takes place within the cocoon previous to pupation.

FIG. 6.—*Chrysopa californica*: Pupa.

As shown by dissections of several cocoons, this resting period, during which the pupa is forming within the larval skin, is from 6 to 9 days in length. Later in the observations it was discovered that one could tell by external indications just when this change

takes place. The larval skin when shed by the pupa is circular in form and is pressed firmly against one end of the pupal case, appearing from without and through the wall of the cocoon (fig. 5) as a dark, almost black, disk.

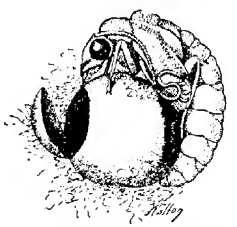

FIG. 7.—*Chrysopa californica:* Pupa freshly emerged from its cocoon.

THE PUPA

The pupa (fig. 6) is formed within a membranous case or cocoon (fig. 5, 7) which is nearly globular in shape, tough but pliable, and inclosed or surrounded by numerous white filaments which hold it in place on the leaf or in some protecting cavity. The cases are often found singly, but when the infestation has been heavy, they may be in groups of a dozen or more. Mr. L. J. Hogg, an assistant, found as many as a dozen or more in a single curled ash leaf, the larvæ having fed on the elm-leaf cluster aphis.

TABLE V.—*Length of the pupal stage of Chrysopa californica at Tempe, Ariz., in 1915*

Cage No.	Date of pupation.	Date adult issued.	Stage length.	Average mean temperature.
			Days.	°F.
78–2	Mar. 17	Apr. 1	15	63
78–3	do.	do.	15	63
78–5	do.	Apr. 2	16	63
79–1	do.	Apr. 4	18	63
79–5	do.	Apr. 7	21	63
80–3	do.	Apr. 2	16	63
80–5	Mar. 18	Apr. 5	18	63
80–6	Mar. 17	Apr. 1	15	63
80–7	do.	do.	15	63
80–10	do.	Mar. 31	14	63
80–11	do.	Apr. 3	17	63
59–2	Oct. 30	Nov. 19	19	57
59–3	Nov. 1	Nov. 20	20	57
59–4	do.	Nov. 21	21	57
59–6	Oct. 31	Nov. 20	20	57
59–7	do.	do.	20	57
59–9	do.	Nov. 21	21	57
59–10	do.	Nov. 19	19	57
59–11	do.	Nov. 22	22	57
59–12	do.	Nov. 19	19	57
59–13	do.	Nov. 22	22	57
59–14	do.	Nov. 20	20	57
Average			18	

The pupal stage in southern Arizona (see Table V) varied from 14 days to 23 days in length, the average being 16⅓ days for March and $20\frac{7}{11}$ days for November.

As has been mentioned, the larva, after constructing the pupal case, which often requires a day's time, may remain several days before pupating. The pupa when formed is curled up as shown in figure 6, with the abdomen closely folded between the large thick wing pads. When ready to change to an adult, the pupa emerges from the cocoon (see fig. 7) through a circular lid, and in from ½ hour to 2 hours the pupal skin is shed and the adult (fig. 1) comes forth. After a few minutes have been allowed for the expansion and drying of the wings, the lacewing fly is ready for flight.

SEASONAL HISTORY AND HIBERNATION

From the writer's observations during the past year (1915) in the Salt River Valley of Arizona, there are at least six generations annually. The first covers the period from about February 15 to March 15, and the remaining generations follow one another every 40 to 45 days from then until late October, either the pupa or adults of the last generation going into hibernation at that time. Adults can be taken throughout the winter months, but eggs have never been secured until the advent of milder weather. Pupæ are often taken during any of the winter months in the Salt River Valley of Arizona, which has a mild winter climate.

NATURAL ENEMIES OF THE LACEWING FLY

It seems that in California (2) the species is commonly attacked by several species of parasites, but no record of any parasite has been obtained during the present study, although abundant material of this lacewing fly was examined. Robber flies have been noted to catch the adults, and certain Hemiptera prey upon the larvæ, but with these exceptions this lacewing fly seems to be quite free and unmolested.[1]

According to the records of the Biological Survey, United States Department of Agriculture, the Western wood pewee (*Contopus richardsonii*) feeds upon the species at Pasadena, Cal.; and at East Bernard, Tex., the nighthawk (*Chordeiles virginianus*) was found feeding upon the species, the stomachs of two birds containing three and six adults, respectively.

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[1] Possibly this is due to the extremely offensive odor thrown off by the adults of all lacewing flies when alarmed.

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RAPE AS MATERIAL FOR SILAGE

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INTRODUCTION

The popularity of rape (*Brassica napus*) as a pasture crop has been steadily increasing since its introduction into this country about 25 years ago. Its value as such is considerable, but its usefulness would be greatly increased if it could be preserved in the silo and used successfully as a succulent feed for the winter months. Attempts to ensile it have, however, evidently been few, perhaps since it has generally been considered too watery for this purpose. The only report of such an attempt which has been found in the literature is from Canada.[1]

In that experiment rape was cut when about 15 inches high and ensiled alone and with an equal weight of corn. When the silage was fed six months later, it was said to have been well preserved, to have had a pleasant odor, and to have been eaten with avidity by cattle. Chemical analyses showed a considerable loss of water and carbohydrates and an increase in nonprotein nitrogen. With the exception of the loss of water, these losses are not much greater than the losses which occur in ensiling the corn plant (*Zea mays*). In that experiment the total loss of dry matter was 26.5 per cent. Weight for weight, however, rape silage was found by analysis to be a much more valuable feeding material than green rape.

In 1914, Evvard, at the Iowa Station,[2] made rape silage in barrels, with and without the addition of common salt (sodium chlorid). The highly salted silage was quite well preserved and had a favorable odor, but was refused by stock. The unsalted silage contained mold and had undergone some putrefactive fermentation, the odor of volatile sulphids being quite evident. The shape of the barrels and the consequent difficulty of excluding air on the settling of the ensiled material were responsible for this putrefaction. This emphasizes the importance of using suitable air-tight containers in making rape silage.

The ideal plant for silage making must contain just sufficient fermentable sugars to furnish enough acids to preserve it. In most respects the corn plant furnishes the most nearly ideal material for silage. The legumes are not ensiled so successfully because the percentage of protein is too high for the amount of sugar, and some putrefaction is likely to

[1] Schutt, F. T. Report of the chemist. Fodders and feeding stuffs. *In* Canada Exp. Farms Rpts., 1904, p. 166–182. 1905.

[2] Unpublished data.

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occur. Rape contains a larger amount of sugars[1] and is therefore likely to develop a high acidity. Rape, in common with other Cruciferae, contains considerable amounts of organic sulphur compounds, which are likely to form disagreeable volatile products if the fermentation progresses too far. For these reasons a mixture of rape and a legume should produce better silage than either alone.

EXPERIMENTAL RAPE SILAGE

The experimental silage was therefore made from rape alone and from mixtures of rape with various other materials, as outlined in Table I, with the purpose of determining the most satisfactory combination. The other plant materials used were alfalfa (*Medicago sativa*), red clover (*Trifolium pratense*), sweet clover (*Melilotus alba*), potato tubers (*Solanum tuberosum*), timothy (*Phleum pratense*), Sudan grass (*Andropogon sorghum, acthiopicus*), sorghum cane (*Sorghum vulgare*), and bluegrass (*Poa pratensis*). The rape used was quite mature but still succulent. The rape leaves were cut off at the main stalk. The entire plant was cut 3 inches from the ground. The alfalfa was cut just before blooming. The corn, Sudan grass, and sorghum cane used were mature. The other plant materials were cut just before maturity. All the forage was cut by a silage cutter into half-inch lengths. The material was tightly packed into glass jars of about 1-gallon capacity, in the same manner as corn silage has repeatedly been made in this laboratory. The jars were closed with metal caps, which were not too tight to prevent the escape of excess gases.

[1] An average air-dry sample contains 5.60 per cent of total fermentable sugars calculated as dextrose.

TABLE I.—*Analyses of rape silage*

Sample No.	Percentage of constituents of sample	Result of inspection.			Class.	Data on 100 c. c. of juice.								Calculated to 100 gm. of dry matter.				
						Water.	Total acidity.		Volatile acidity.			Alcohol.	Amino nitrogen.					
		Appearance.	Odor.	Taste.			N/10 solution.	Calculated as lactic acid.	N/10 solution.	Calculated as acetic acid.	Fixed acidity (by difference) calculated as lactic acid.			Total acidity calculated as lactic acid.	Volatile acidity calculated as acetic acid.	Fixed acidity (by difference) calculated as lactic acid.	Alcohol.	Amino nitrogen.
						Per ct.	*C. c.*	*Gm.*	*C. c.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
1	Rape entire 100	Good	Pleasant	Sour	B	84.5	275	2.475						13.572				
2	Rape leaves 100	do	do	Very sour	C	86.9	298	2.682	76.8	0.461	1.989	[a] 0.246	0.281	16.452	2.827	12.213	0.220	1.724
3	Rape 80, alfalfa 20	do	Aromatic	do	B	84.7	253	2.268						12.328				
4	Rape 60, alfalfa 40	do	Unpleasant	Disagreeable [b]	E	83.3	189	1.701						8.595				
5	Rape 40, alfalfa 60	do	Aromatic	Good, but sour	A	84.1	227	2.034						10.602				
6	Rape leaves 80, alfalfa 20	do	Sauerkraut	Very sour	C	85.8	250	2.250	128.2	.769	1.093	[a] .060	.268	13.554	4.634	6.666	.361	1.614
7	Rape leaves 60, alfalfa 40	do	do	Good, but too sour	B	85.5	232	2.088						12.276				
8	Rape leaves 40, alfalfa 60	do	Aromatic	Favorable	A	82.2	269	2.421						11.151				
9	Rape leaves 90, cane sugar 10	do	do	Pleasant, but very sour	C	81.8	342	3.078	69.2	.415	2.457	[a] .036 [b] .570	.280	13.860	1.870	11.052	[17.954 17.838]	1.252
10	Rape leaves 90, molasses 10	Dark color	Agreeable	Too sour	C	84.3	391	2.709	48.8	.293	2.268	[a] .011		14.562	1.574	12.204	10.812	
11	Rape 90, molasses 10	do	do	do	C	82.1	351	3.159						13.490				
12	Rape leaves 80, molasses 20	Dark	Like molasses	Sour	C	82.1	345	3.105						14.238				
13	Rape 80, starch 20	do	Starchy (slimy)	do	D	75.4	244	2.196						6.453				
14	Rape leaves 80, starch 20	do	do	Less sour	D	77.2	123	1.107						3.744				
15	Rape leaves 60, potatoes 40	Good	Sour	Not bad	C	84.7	188	1.692						9.342				
16	Rape leaves 80, corn grain 20	do	Good, but sour	Very sour	C-D	69.9	355	3.195						7.497				
17	Rape leaves 60, whole corn plant 40	do	Pleasant	do	C	79.4	342	3.078						11.880				
18	Rape 60, corn grain 40	do	Agreeable	Good, but sour	B	58.3	412	3.978	96.5	.543	3.159	[c] .424	.360	5.362	.760	3.319	.593	.643

[a] By aeration.

[b] Possibly accidental contamination.

[c] By distillation.

TABLE I.—*Analyses of rape silage*—Continued

Sample No.	Percentage of constituents of sample.	Result of inspection.			Class.	Water.	Data on 100 c. c. of juice.							Calculated to 100 gm. of dry matter.				
		Appearance.	Odor.	Taste.			Total acidity.		Volatile acidity.			Alcohol.	Amino nitrogen.	Total acidity calculated as lactic acid.	Volatile acidity calculated as acetic acid.	Fixed acidity (by difference) calculated as lactic acid.	Alcohol.	Amino nitrogen.
							N/10 solution.	Calculated as lactic acid.	N/10 solution.	Calculated as acetic acid.	Fixed acidity (by difference) calculated as lactic acid.							
						Per ct.	*C. c.*	*Gm.*	*C. c.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
19	Rape leaves+100 gm. of calcium carbonate.	Dark	Disagreeable	"Rotten"[a]	F		81	0.729										
20	Rape+water	do	Strong	Disagreeable	E	90.7	151	1.359						11.439				
21	Rape leaves + water.	do	do	Disagreeable, sour.	E	89.5	155	1.395						11.910				
22	Rape leaves 60, red clover 40.	Good	Aromatic and pleasant.	Very palatable	B	84.2	272	2.448	74.6	0.442	1.785	[b] 0.147	[b] 224	12.174	2.186	8.838	0.727	1.109
23	Rape leaves 60, sweet clover 40.	do	Good (suggests sweet clover).	Bitter, sour	C	84.1	272	2.448						12.951				
24	Rape leaves+lactic-acid culture.	Dark	Rather disagreeable.	Unpleasant, biting.	D	86.3	224	1.993	72.9	.435	1.339			12.564	2.678	8.550		
25	Rape leaves 60, blue grass 40.	Good	Good	Agreeable, but sour.	C	77.3	321	2.889						9.829				
26	Rape leaves 60, timothy 40.	do	Aromatic	Sour	C	79.9	346	3.069						14.472				
27	Rape 60, Sudan grass 40.	do	Aromatic like corn silage.	Slightly acid	B	74.1	340	2.970						8.478				
28	Rape leaves 60, sorghum cane 40.	do	do	Sour	C	74.4	339	2.961						8.604				

[a] Crystals of calcium salts present.

[b] By aeration.

The jars were opened four months after filling and the condition, appearance, odor, and taste of the silage noted. (See Table I.) With very few exceptions it was in a perfect state of preservation, of excellent texture and color, with a pleasant, somewhat aromatic odor, and generally of an agreeable taste, though quite sour. It was succulent without being too moist, even though it had been made in a tightly sealed jar, with almost no opportunity for the evaporation of water. In order to ascertain its palatability to swine, a representative number of the various mixtures and some of the pure rape silage were fed to three lots of pigs. At first the animals, which were on a ration consisting mainly of corn and tankage, tasted the silage rather hesitatingly and seemed surprised by the sourness, but kept at it until they had eaten it all, appearing to enjoy its succulency. On a second trial, three days later, the same animals ate it with great relish. Only one sample of those tried, a rape-molasses mixture, was refused by the animals. In nearly every case after eating this silage they went to the corn self-feeders. An extensive feeding experiment to determine the effect of feeding rape silage upon the growth and well-being of swine is contemplated.

CHEMICAL EXAMINATION OF SILAGE SAMPLES

The data from the chemical examination of the samples are shown in Table I. The juice was pressed out from the silage, and samples were taken from the juice with pipettes. This method has been used with corn silage and is a quite accurate and excellent comparative method for quickly determining the character of a sample of silage. Estimations of the total acidity and moisture content were made on all samples, and estimations of volatile acidity, alcohol, and amino nitrogen on a few representative samples, according to the following methods. (See Table I.)

TOTAL ACIDITY.—Ten c. c. of juice were diluted to about 500 c. c. with carbon-dioxid-(CO_2)-free water, and titrated with decinormal barium-hydroxid solution in the presence of phenolphthalein till a distinct pink appeared by reflected light against a white background.

VOLATILE ACIDITY.—Fifty c. c. of juice were diluted to 100 c. c. with carbon-dioxid-free water and distilled with a current of carbon-dioxid-free steam. To hasten the liberation of volatile acids and alcohols, 100 gm. of sodium chlorid were added to the juice. About 500 c. c. of distillate were titrated with baryta water in the presence of phenolphthalein.

ALCOHOL.—Distillation method: The distillate from the volatile-acid determination was neutralized with baryta water (solid phenolphthalein being added) and concentrated by repeated distillation with sodium chlorid.[1]

About 50 c. c. of alcohol solution were oxidized [2] in a pressure flask in a boiling water bath for 30 to 40 minutes, and the volatile acids then distilled off four or five

[1] Bacon, R. F. Detection and determination of small quantities of ethyl and methyl alcohol and of formic acid. U. S. Dept Agr. Bur. Chem. Circ. 74, 8 p. 1911.

[2] The oxidizing solution used was made up in the following proportions: 10 gm. $K_2Cr_2O_7$, 20 gm. H_2SO_4, 70 gm. water.

times, with additions of carbon-dioxid-free water. The total alcohols found were calculated as ethyl alcohol.

Aeration method: In this method a current of air was drawn through the alcohol solution, which was saturated with ammonium sulphate, into concentrated sulphuric acid. The sulphuric-acid solution was then oxidized with potassium-dichromate solution and distilled as before.

AMINO NITROGEN.—The amino nitrogen was determined on the diluted juice with the Van Slyke apparatus.[1]

MOISTURE.—The moisture content was determined by heating a sample of about 100 gm. in an oven at 100° C.

DISCUSSION

The determinations of total acidity, volatile acidity, total alcohols, and amino nitrogen furnish a measure of the most characteristic changes which take place in silage fermentation and a partially complete picture of the character of the fermentation and the character of the silage, as nearly as chemical analysis can show. This, the ordinary estimations of crude protein, fiber, ether extract, and ash fail to do. The amount of amino nitrogen is, of course, of comparative value only, but it shows the degree of hydrolysis of protein. Unfortunately in this case no figures are now available for the amino nitrogen of green rape. The results given in Table I, however, indicate that the degree of hydrolysis of protein was nearly the same in each sample upon which this determination was made. The total acidity was quite similar in each of the samples which were classed "A" and "B." The total acidity of the silage juice in most cases is no higher than the average acidity of corn-silage juice. The average of analyses on 100 c. c. of juice of several samples of normal corn silage is as follows:

Total acidity	271 c. c. of *N*/10 solution.
Volatile acidity	91 c. c. of *N*/10 solution.
Alcohol	0.312 gm.
Amino nitrogen	0.109 gm.

The explanation of the very sour taste of rape silage may lie in the fact that it has a much higher water content than corn silage and thus affects the nerves of taste more quickly. A considerable amount of sulphates was found in one sample, but the presence of any free mineral acid could not be demonstrated. The volatile acidity seemed to vary more widely, with varying experimental conditions. The alcohol content was probably small in all cases where there was no addition of sugar. In two cases of silage with added sugar or molasses Table I shows that an abnormally large amount of alcohol was found. This, as well as the increased acidity, militates against the addition of molasses to silage materials. It is very probable that the excess alcohol was formed after the maximum acidity had been reached and the yeasts had gained the ascendancy.

[1] Van Slyke, D. D. The quantitative determination of aliphatic amino groups, II. *In* Jour. Biol. Chem., v. 12, no. 2, p. 275–284, 1 fig., 1 pl., 1 tab. 1912.

The classification of the samples as to general silage quality (A, B, C, etc.) is necessarily approximate. All the samples, however, could be classed as "good silage," except those rated below "D." Those containing fibrous material, such as sorghum cane, Sudan grass, timothy, and corn plant, would be useful for cattle, but would not be as good feed for swine as pure rape silage, or the alfalfa, red clover, potato, or corn-grain mixtures. The silage made from the entire rape plant was quite similar to that made from the leaves. However, for swine too much fiber is objectionable.

The mixtures of rape with legumes are perhaps best, from the standpoint of feeding as well as that of the quality of the silage. The rape improves the mixture, in that it supplies the necessary fermentable carbohydrates, which apparently are deficient in amount in the legume. In this connection it may be noted that since legume silage is not entirely satisfactory, it may be greatly improved by adding 20 per cent or more of rape, which would supply the necessary sugars. On general considerations the indications are that this sort of silage should be useful for either cattle, sheep, or swine. Practical farmers have sowed rape in the cornfield at the time of the last cultivation, it later being ensiled with the corn. This mixed silage has been fed to cattle with apparently good results.

SUMMARY

(1) Rape was successfully ensiled in glass jars, alone and in mixtures with other materials.

(2) Excepting one or two mixtures, this silage was palatable to swine.

(3) Chemical examination of the samples showed the acidity and alcohol content to be comparable in most cases to that of corn silage.

(4) A mixture of rape and a legume produces the best quality of silage.

EFFECT OF AUTOLYSIS UPON MUSCLE CREATIN

By RALPH HOAGLAND, *Senior Biochemist*, and C. N. MCBRYDE, *Senior Bacteriologist, Biochemic Division, Bureau of Animal Industry*[1]

INTRODUCTION

The question as to the relation between muscle creatin and urinary creatinin is one which has been the subject of considerable investigation, particularly during the past few years. The importance of this problem lies in the fact that it is now quite clearly established that the creatinin excreted in the urine with a creatin-creatinin-free diet, is an accurate measure of endogenous metabolism. In the mammalian family, creatin is found chiefly in the striated muscular tissue, and to a lesser extent in other tissues and in fluids. The anhydrid creatinin is present in very small quantities. Since the amount of creatinin excreted in the urine is an accurate measure of tissue metabolism and since creatin is a normal constituent of muscular tissue, and since also there is a close chemical relationship between the two compounds, the natural supposition is that urinary creatinin is derived from muscle creatin. This may be said to be the generally accepted view, and it is supported by considerable experimental evidence; yet, on the other hand, certain investigators have obtained results which do not appear to support this theory.

The question as to where creatinin is formed in the body is another problem concerning which there is considerable lack of agreement. In the light of our present knowledge on the subject it must be admitted that the method and the place of production of creatinin in the body have not been clearly established.

In the course of a series of autolytic experiments with lean beef, carried on in connection with investigations concerning changes taking place in beef in cold storage, certain changes were noted in the creatin and creatinin content of the muscles which appear to throw some light on the question as to the source and method of production of creatinin. The results of these observations are offered as a contribution to our knowledge of the subject.

PREVIOUS AUTOLYTIC EXPERIMENTS

Gottlieb and Stangassinger (3)[2] carried on an extensive series of autolytic experiments with various organs, tissues, and fluids of dogs, cats, and calves, using toluol as an antiseptic. As a result of their studies these

[1] The authors desire to extend their thanks to Mr. W. C. Powick for assistance rendered in connection with the analytical work reported in this paper.

[2] Reference is made by number to "Literature cited," p. 546.

authors came to the following conclusions: (1) Muscles and other tissues produce creatin in the early stages of autolysis; (2) natural and added creatin are changed in part to creatinin, owing to the action of dehydrating ferments; (3) creatin and creatinin are in part destroyed as autolysis progresses, owing to the action of ferments which they name "kreatase" and "kreatinase."

The work of these authors, so far as changes in free creatinin are concerned, is open to criticism on account of the method which they used for the determination of this constituent. The extracts were concentrated nearly to dryness on a steam bath, the solutions having been neutralized by the addition of barium carbonate. It is now recognized that such a method of concentrating a solution containing creatin will convert a part of that base into creatinin. For these reasons the work of Gottlieb and Stangassinger concerning the production of free creatinin during autolysis must be regarded as of doubtful value.

Stangassinger (9) studied the action of autolyzing body tissues and fluids upon added creatin and the effect of various chemicals and conditions upon the rate and extent of the reaction. Blood, kidneys, livers, and lungs of dogs were used in the experiments. The so-called dehydrating ferments kreatase and kreatinase were found to be most active in weak acid solutions, and toluol had the least retarding action of all the antiseptics used. Protoplasmic poisons checked the action of the ferments. Creatin was formed in the early stages of the autolysis of liver and blood, the material from well-fed animals containing larger amounts of creatin-forming material than that from hungry dogs. Liver extract destroyed added creatinin in appreciable quantities.

This author's findings concerning changes in free creatinin are open to the same criticisms as those made of the work of Gottlieb and Stangassinger (3).

Mellanby (4) carried on autolytic experiments with various tissues, but was unable to confirm in any respect Gottlieb and Stangassinger's findings (3) concerning the effect of autolysis of tissues upon creatin and creatinin. A careful examination of Mellanby's article indicates that his conclusions should not be taken too seriously. For example, rabbit muscle was autolyzed, under strictly aseptic conditions, for five days at 37° C., and at the end of that time no free creatinin could be detected. If it could not then be detected, it certainly could not be found in the author's other experiments, in which autolysis was carried on under less favorable conditions.

Rothmann (7) carried on a series of autolytic experiments in reply to Mellanby's criticism (4) of Gottlieb and Stangassinger's work (3). The work was conducted under strict bacteriological control and it was found that the liver, kidney, and blood of dogs destroyed appreciable quantities of creatin. He admits the correctness of Mellanby's criticism of Gottlieb and Stangassinger's method for the determination of free

creatinin, stating that in the operation creatin was probably changed in part to creatinin. However, using Mellanby's method for the determination of free creatinin, he found that liver and kidney extracts converted appreciable quantities of creatin into creatinin.

Pekelharing and Van Hoogenhuyze (6) found fairly marked increases in the creatin content of muscles on the completion of rigor mortis and heat rigor.

Rowe (8) carried on autolytic experiments with the parathyroids and adrenals of sheep and found that, in a marked degree, these tissues had the property of destroying added creatin. Thyroid extract destroyed 71 per cent of the added creatin in 48 hours and adrenal extract destroyed 69 per cent in 72 hours.

Myers and Fine (5) studied the effect of autolysis upon the creatin and creatinin content of various tissues and fluids of vertebrate animals. Very marked increases were noted in the creatinin content of all the materials examined after autolysis. Human blood and rabbit liver showed marked gains in the total creatinin. In the case of dog muscle an appreciable decrease in total creatinin was noted. The authors are of the opinion that muscular tissue is the site of creatinin formation.

THE PRESENT EXPERIMENTS

Two series of autolytic experiments were carried on: One under aseptic conditions; the other with the use of antiseptics. It is generally recognized that the aseptic method is to be preferred, so far as the value of the results is concerned; but owing to the extreme care required in carrying on an autolytic experiment under aseptic conditions, the antiseptic method is commonly employed. In these investigations the antiseptic method was used simply as a check against the aseptic method, and for the purpose of comparison.

ASEPTIC AUTOLYSIS EXPERIMENTS

A prime steer was slaughtered at a local abattoir by the usual methods under the personal supervision of one of the authors. It was, of course, impossible to carry out the operation of skinning under strictly aseptic conditions, so the chief aim was to make this operation as cleanly as possible. The entire carcass was first wet down to prevent the dissemination of dust particles. The carcass was kept suspended while it was being skinned and was not allowed to come into contact with the floor, which had also been washed to prevent dust from rising. In skinning the carcass, knives were used which had been dipped in boiling water, and they were again dipped from time to time in the boiling water. As soon as the skin was removed one of the hindquarters was wound with gauze which had been wrung out in a solution of mercuric chlorid (1:1,000); then it was separated from the body and completely enveloped in the

gauze. The hindquarter was next wrapped in dry cheesecloth and heavy paper and transported at once to the laboratory by motor truck, the trip requiring less than an hour.

METHOD OF TAKING SAMPLES

To obtain relatively large and aseptic samples of meat such as were used in these experiments is not an easy matter, and extreme care had to be taken to prevent bacterial contamination. After several failures samples free from bacteria were obtained in the following manner: At the laboratory the hindquarter was transferred at once to a special inoculating room about 10 feet square. The walls and floors of this room had been previously washed with the mercuric-chlorid solution. A special canopy ceiling consisting of cheesecloth tacked on a light frame had been placed in the room at the height of about 10 feet, and this was sprayed with a solution of liquor cresolis compositus just before taking the samples. The floor and walls were also sprayed at the same time with the compound cresol solution and were damp while the samples were being taken, the idea being to have the floor, walls, and ceiling moist, so that any floating dust particles would stick to them.

For taking the samples a number of large, heavy-bladed scalpels and long dissecting forceps were used; these had been sterilized and wrapped in cotton. Large plugs of meat, approximating 3-inch to 4-inch cubes, were cut from the muscular tissue, avoiding connective tissue and fat as much as possible. These plugs, weighing from 274 to 512 gm., the average being 377 gm., were immediately transferred to sterile crystallizing dishes fitted with deep glass covers. In cutting out the plugs the line of incision was first thoroughly seared with a hot spatula. Then a light cut was made through the outside to the depth of about 0.5 cm. and the knife used for the incision was laid aside. A second sterile knife was then used for continuing the deeper incision. This was done in order not to carry in any of the mercuric-chlorid solution which might have adhered to the outside. The outer or exposed portions of the meat samples were always trimmed away to the depth of at least half an inch in order to eliminate those portions which had come in contact with the bichlorid gauze. Thirty-three samples were taken in this manner.

The dishes containing the samples were weighed, the covers sealed with adhesive tape, and over the tape were placed strips of tin foil. This was done for the double purpose of preventing evaporation and the possibility of bacterial contamination from the outside.

BACTERIOLOGICAL CONTROL OF SAMPLES

The dishes containing the meat samples were placed in the incubator and carefully watched from day to day for evidence of bacterial growth.

Twenty-four of the thirty-three samples showed bacterial contamination upon incubation—that is, visible bacterial growths developed on

the moist surface of the samples, which furnished a good culture medium for bacterial growth. These samples were, of course, rejected. The remaining samples showed no visible bacterial growths upon incubation and were removed from the incubator one at a time after intervals ranging from 7 to 100 days and subjected to a bacteriological examination.

In examining the samples bacteriologically, aerobic and anaerobic cultures were first made from the exuded juice. With sterile instruments bits of muscular tissue were then cut from the outside of the samples and used for cultures. The samples, which, as before stated, consisted of large rectangular pieces approximating 3-inch cubes, were then cut in two with sterile instruments and cultures made by taking bits of the muscular tissue from the center of the samples. A half dozen or more cultures were taken from each sample. Smear preparations were also made from the exuded juice and from the outer and inner portions of the samples and were stained for bacteria.

Upon bacteriological examination nine of the samples were passed as sterile, there being no growths in any of the cultures made from these samples and the smear preparations being negative. These samples were then subjected to chemical analysis (Table I).

The fact that so large a proportion of the samples, 24 out of 33, or about 72 per cent, developed bacterial growths goes to show how difficult it is to obtain sterile samples of meat.

METHODS OF CHEMICAL ANALYSIS

After having taken the samples of muscular tissue for incubation the remainder of the quarter of beef was placed in cold storage at 33° F. for 17 hours, when a composite sample of the lean meat was taken for analysis. Analytical work was started about 24 hours after the slaughter of the animal.

All samples of meat, both fresh and after incubation, were finely ground, placed in glass jars, tightly sealed, and analytical work was started promptly.

Moisture and fat determinations were made on all samples. Moisture was determined by drying the material in vacuo over sulphuric acid, and fat was determined in the dry residue by extraction with ether.

PREPARATION OF EXTRACT.— A 0.9 per cent solution of sodium chlorid, saturated with thymol, was used as a solvent. One hundred gm. of the finely ground tissue were macerated in a mortar with the salt solution until a mixture of uniform consistency was obtained. The material was then transferred to a 2-liter volumetric flask, made to volume with the salt solution, and shaken at intervals during a total extraction period of 24 hours. The mixture was then filtered and analytical work begun immediately. Extractions were made in duplicate and the work was carried on in a refrigerated room at a temperature of about 35° F.

ACIDITY was determined by titrating 50 c. c. of the filtered extract against standard sodium-hydroxid solution, using phenolphthalein as an indicator. The results are calculated in terms of lactic acid.

TOTAL CREATININ was determined according to the method of Folin as modified by Emmett and Grindley (1, p. 515). The results are calculated in terms of creatinin.

FREE CREATININ was determined essentially according to the method of Folin (2). Standard creatinin solutions were made from creatinin which had been standardized against *N*/2 potassium bichromate. With close attention to all details this method was found to give very satisfactory results.

Table I shows the changes in the creatin and creatinin content of lean beef autolyzed under aseptic conditions for periods ranging from 7 to 100 days.

TABLE I.—*Changes in creatin and creatinin content of muscle during aseptic autolysis at 37° C.*

Serial No.	Incubation period.	Percentage of acid as lactic.	Percentage of total creatinin.	Percentage of free creatinin.	Percentage of creatin calculated as creatinin.	Percentage of total creatinin as free creatinin.
	Days.					
109		3.15	1.73	0.036	1.694	2.08
110	7	3.03	1.97	.422	1.548	21.42
111	14	3.15	1.91	.603	1.307	31.57
112	21	3.16	1.91	.706	1.204	36.96
113	28	4.75	1.63	.756	.774	46.58
120	42	4.53	1.64	.761	.879	46.40
121	64	4.53	1.55	.670	.880	43.23
122	77	5.02	1.62	.742	.878	45.80
124	93	4.74	1.54	.707	.833	45.91
125	100	4.76	1.68	.728	.952	43.33

Changes in total creatinin are fairly marked. Samples incubated for 7, 14, and 21 days show increases in total creatinin amounting to 0.24, 0.18, and 0.18 per cent, respectively. Samples incubated for longer periods, ranging from 28 to 100 days, show appreciable losses in total creatinin varying from 0.19 to 0.05 per cent. On the whole, these data show first an increase in total creatinin and later a decrease, the increases being somewhat larger than the decreases.

The changes in the free creatinin are very marked. The fresh material contains 0.036 per cent of free creatinin, while the sample incubated 7 days contains 0.422 per cent, an actual increase of 0.386 per cent, or a relative increase of 1,722 per cent. Samples incubated for 14, 21, 28, and 42 days show further increases in free creatinin, but the rate of increase is less rapid with each succeeding period. The maximum percentage of free creatinin, amounting to 0.761 per cent, is found in case of the sample incubated 42 days. This is an actual increase of 0.725 per

cent of creatinin as compared with the fresh material. Samples incubated for periods ranging from 64 to 100 days show slight and irregular decreases in free creatinin as compared with the sample incubated 42 days.

The creatin content of the samples, which is calculated by subtracting the percentage of free creatinin from that of total creatinin, shows decreases which correspond to the increases in free creatinin.

The relation between the free creatinin and total creatinin is of special interest. The fresh material contains 2.08 per cent of the total creatinin in the form of free creatinin, while in case of the sample incubated for 7 days the percentage has increased to 21.42. The increases in succeeding periods are less rapid, until a maximum increase is reached in case of the sample incubated 42 days, which contains 46.40 per cent of the total creatinin in the form of free creatinin. However, practically the maximum increase is reached in case of the sample incubated 28 days in which 46.38 per cent of the total creatinin is in the form of free creatinin.

These results show that under the conditions of the experiment an equilibrium is established between the creatinin and creatin. These findings confirm in a remarkable degree results obtained by Myers and Fine (5) in their work with pure solutions of creatin and of creatinin. They incubated solutions of the individual bases for a total period of 337 days, and determined free and total creatinin in each of the solutions at intervals. In case of the solution of creatin, it was found that there was a gradual change of creatin into creatinin until at the end of the period an equilibrium had been established with 44.45 per cent of the total creatinin in the form of free creatinin. In case of the solution of creatinin the change was in the other direction, there being a decrease in creatinin and an increase in creatin, until at the end of 337 days an equilibrium had been established with the relative proportions of creatin and creatinin identical with those noted above.

It is not to be inferred from these findings that the changes which took place in the creatin and creatinin content of muscular tissue during autolysis are entirely natural changes of one base into the other. In case of the autolytic experiments with muscle, practically the maximum change of creatin into creatinin had taken place at the end of 28 days, and nearly half of the total change had taken place in 7 days.

In Myers and Fine's experiments (5) with a solution of pure creatin only 9 per cent of the total creatinin was present in the form of free creatinin at the end of 13 days, and after 53 days only 29 per cent. In our autolytic experiments with muscle, on the other hand, 25.41 per cent of the total creatinin was in the form of free creatinin at the end of 7 days, and 46.31 per cent at the end of 28 days.

It is very evident that the rate of change of creatin into creatinin during the autolysis of beef muscle was greatly accelerated by some agent. The acids in the meat may have facilitated the change in some degree;

but the facts seem to indicate that in considerable part, at least, the change of creatin into creatinin during the autolysis of beef muscle was caused by enzym action.

ANTISEPTIC AUTOLYSIS EXPERIMENTS

Muscular tissue, consisting of the pillar of the diaphragm, was obtained from the carcass of a steer immediately after slaughter. The meat was freed from visible fat and connective tissue and finely ground. Thirty-five gm. of meat were weighed into a mortar with 20 gm. of sand, and 50 c. c. of a 0.9 per cent solution of sodium chlorid were added. The tissue was ground to a mass of uniform consistency and then transferred to a 250 c. c. Erlenmyer flask with the aid of 100 c. c. of the salt solution, and the flask was stoppered with a rubber stopper. Sixteen samples were prepared in this manner. After all the samples had been prepared, 2 c. c. each of chloroform and toluol were added to each flask which was then thoroughly shaken. Fourteen of the flasks were then placed in an incubator where they were held at 37° C. for various periods of time. The flasks were shaken daily to insure saturation of the solutions with the antiseptics. Two flasks were placed in a cold-storage room at a temperature of 34° F. and shaken at intervals for a period of 24 hours for the purpose of determining the creatin and creatinin in the fresh material.

BACTERIOLOGICAL CONTROL OF SAMPLES

Before adding the antiseptics and before incubation, bacterial counts were made of three of the samples, Nos. 2, 8, and 17, which had been prepared as described above. In making the counts, 0.5 c. c. and 1 c. c. portions of the samples were withdrawn with sterile pipettes and added to tubes of melted agar which were immediately poured into Petri dishes and incubated. The bacterial counts on the three samples were as follows:

Sample 2.....2,116 bacteria per cubic centimeter.
Sample 8.....1,480 bacteria per cubic centimeter.
Sample 17.....1,584 bacteria per cubic centimeter.

The samples were prepared one at a time in the order in which they were numbered—that is, from 1 to 18—and the higher bacterial count in the case of sample 2 is probably due to the fact that this flask was the first one prepared and remained standing for several hours at room temperature, thus giving time for bacterial multiplication before the counts were made. The three counts were made in order to give some idea of the average number of bacteria in the samples before adding the antiseptics and before incubation.

The samples were removed from the incubator for chemical analysis at the intervals given in Table II. In testing the samples bacteriologically, two portions of 1 c. c. each were removed with sterile pipettes and

agar plates made therefrom. In withdrawing the portions for cultures the point of the pipette was introduced well below the surface of the liquid so as to avoid drawing up any of the toluol which floated on the surface. The chloroform, being heavy, settled to the bottom.

In order to avoid carrying over any of the toluol on the pipettes, the ends of the pipettes were washed with sterile, distilled water before their contents were delivered into the agar tubes. A single colony was observed in one of the plates made on the fourth day and single colonies were observed in each of the plates made on the eighth day, but after this the plates remained sterile.

The absence of bacterial development in the plates may have been due to inhibition of growth by small amounts of the antiseptics dissolved in the meat infusion rather than to actual destruction of the organisms present. However, the results seem to afford ample evidence that there was no bacterial multiplication in the samples during the course of the experiment.

CHEMICAL STUDIES

Moisture was determined in the fresh material for the purpose of correcting for the volume of water in the meat.

Creatin and creatinin were determined in the filtered extracts from the various samples according to the methods of Folin, as previously noted (2).

Table II shows the changes in the creatin and creatinin content of muscular tissue from the ox incubated at 37° C. in the presence of antiseptics for periods ranging from 2 to 84 days.

TABLE II.—*Changes in creatin and creatinin content of beef muscle during antiseptic autolysis at 37° C.*

[Expressed as percentages of fresh material.]

Serial No.	Incubation period.	Percentage of total creatinin.	Percentage of free creatinin.	Percentage of creatin calculated as creatinin.	Percentage of total creatinin as free creatinin.
1		0.28	0.0047	0.2753	1.67
2	48 hours	.29	.0140	.2760	4.78
4	95 hours	.28	.0195	.2605	7.07
5	7 days	.29	.033	.257	11.26
6	14 days	.29	.049	.241	16.65
7	21 days	.28	.060	.220	21.09
8	28 days	.27	.067	.203	25.09
9	35 days	.28	.080	.200	28.12
10	42 days	.28	.107	.173	37.61
11	49 days	.28	.098	.182	35.53
12	56 days	.28	.101	.179	36.61
13	63 days	.29	.109	.181	37.19
14	70 days	.29	.112	.178	38.21
15	77 days	.28	.113	.167	30.70
17	84 days	.29	.113	.177	38.53

There are practically no changes in the total creatinin in contrast to the fairly marked changes in this constituent noted in case of the aseptic autolytic experiment. This fact does not indicate that the changes in total creatinin content observed in case of the aseptic autolytic experiment are in error, but rather that the antiseptics used in the second experiment probably prevented the change.

There is a marked increase in free creatinin during the course of the experiment, the increase taking place most rapidly in the early stages of the incubation period, and less rapidly toward the end of the experiment, until finally there was practically no change.

On account of the different bases of calculation, these data can not be compared directly with similar data obtained in case of the aseptic autolytic experiment. However, the general trend of the change in free creatinin is the same in each experiment. In the first experiment a maximum production of free creatinin was reached in 42 days, in the second experiment in 84 days.

Creatin shows decreases corresponding to the increases in creatinin. The data showing changes in the relation of free creatinin to total creatinin indicate most clearly the changes in these constituents during the course of the experiment. The transformation of creatin into creatinin takes place most rapidly during the first 24 hours, and the rate of change steadily decreases during the course of the experiment, until at the end of 77 days a maximum change is reached, the free creatinin then constituting 39.70 per cent of the total creatinin. It is possible that if the experiment had been continued for a much longer time a larger proportion of creatin would have been converted into creatinin. These data confirm the changes of creatin to creatinin observed in the case of the aseptic autolytic experiment, and also the fact that the total extent of the change is limited. In the first experiment a maximum change of 46.40 per cent of total creatin, calculated as creatinin, to creatinin was observed, while in the second experiment the total change amounted to 39.70 per cent.

The results obtained in the antiseptic autolytic experiment confirm those obtained in the experiment conducted under aseptic conditions, both as regards change of creatin into creatinin, and in that the total extent of the reaction is limited, but do not confirm those showing first an increase and later a decrease in total creatinin.

DISCUSSION OF RESULTS

The results of the experiments reported in this paper show very clearly the transformation of muscle creatin into creatinin during autolysis. To a very considerable degree this transformation must be regarded as due to the action of enzyms.

These findings are substantiated by the work of Gottlieb and Stangassinger (3), Stangassinger (9), Rothmann (7), Rowe (8), and Myers and

Fine (5). Mellanby (4) obtained contrary results; but, as has already been noted, a careful examination of his paper indicates something wrong with his work, since he was unable to detect creatinin under conditions in which it was undoubtedly present. His findings should not be taken too seriously. The ability of autolyzing muscular tissue, as well as of other body tissues, to transform creatin into creatinin seems to be quite clearly established.

In the aseptic autolytic experiment there was first an increase in total creatinin and later a decrease as compared with the amount present in the fresh material, while in the experiment carried on under antiseptic conditions there was practically no change. As has been previously noted, it does not follow that the results of the first experiment are in error, but it is possible that in the second experiment the presence of antiseptics prevented these changes in creatin.

A brief examination of the work of previous investigators on this point may throw some light on the question. Gottlieb and Stangassinger (3) observed at first an increase and later a decrease in the total creatinin content of muscular and other body tissues and fluids on autolysis. Stangassinger (9) found an increase in the total creatinin content of blood and liver of dogs and later a decrease in the total creatinin content of the liver. Rothmann (7) found that extracts of the liver and kidney of dogs destroyed added creatinin in a marked degree, and that there was a marked increase in the creatin content of the portal blood of a dog. Rowe (8) observed that extracts from the parathyroid and adrenal glands of sheep destroyed added creatin. Pekelharing and van Hoogenhuyze (6) found an increase in the creatin content of the muscles of dogs after rigor mortis and heat rigor. Myers and Fine (5) found an increase in the total creatinin content of autolyzing human blood and rabbit liver, and a decrease in the total creatinin content of dog muscle. On the whole, the work of these investigators confirms our findings concerning changes in the total creatinin content of beef muscle during aseptic autolysis.

In keeping with the results obtained by Pekelharing and van Hoogenhuyze (6) concerning the effects of rigor mortis upon the creatin content of muscular tissue, it seems very probable that the increase in the total creatinin content of the muscle in our aseptic autolytic experiment was due to the changes accompanying rigor mortis. While analytical work was started 24 hours after the slaughter of the animal, at which time rigor was assumed to be complete, yet in a study of the effects of autolysis upon the soluble muscle proteins changes were observed which indicated that it was not complete at that time.

The establishment of an equilibrium between creatin and creatinin in solutions of the individual bases, as observed by Myers and Fine (5), and our finding as to the establishment of a similar relation between creatin and creatinin in autolyzing muscular tissue, is a matter of more

than passing importance. It denotes, first, that creatin is readily converted into creatinin in pure solution, and, second, that in autolyzing muscular tissue the rate of reaction is very greatly accelerated, but that the total extent of the change is the same in either case. The more rapid change of creatin into creatinin in the autolyzing tissue may safely be assumed to be due, in large part, at least, to enzym action. This conforms to our idea as to the catalytic nature of enzyms. The gradually reduced rate of change of creatin to creatinin during autolysis is in conformity with the law of mass action. These observations are of more interest as regards the chemical relationship of the two substances than on account of their physiological relationship, since in the animal body any change of creatin to creatinin is accompanied with the rapid removal of the creatinin, so that, so far as this factor is concerned, the change always takes place at its maximum velocity. The clear establishment of the fact that muscular tissue has the power in a marked degree of converting creatin into creatinin must be regarded as having an important bearing upon the formation of creatinin in the body. Without going into a discussion of other investigations bearing upon this subject, it may be said that there is much evidence in support of the theory that muscle creatin is the source of urinary creatinin, with a creatin-creatinin-free diet, and considerable evidence to the effect that in part, at least, the transformation of creatin into creatinin takes place in the muscular tissue.

SUMMARY

The results of the investigations reported in this paper concerning the effects of autolysis upon the creatin and creatinin content of muscular tissue of the ox may be summarized as follows:

(1) Muscular tissue has in a marked degree the property of converting creatin into creatinin.

(2) In the course of autolysis an equilibrium is finally established between creatin and creatinin.

(3) Muscular tissue appears to have in an appreciable degree the ability both to produce and to destroy creatinin.

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